ABSTRACT

Mouse polyomavirus (MPyV) is a non-enveloped DNA tumor virus, which replicates in the host cell nucleus. MPyV enters cells by receptor-mediated endocytosis and its subsequent transport towards the nucleus requires acidic environment of endosomes and intact microtubules, which are important for virus delivery to endoplasmic reticulum (ER). In ER, capsid disassembly and uncoating of viral genome take place. The mechanism of subsequent translocation of viral genome from ER into nucleoplasm is still only poorly understood process with predicted involvement of cellular factors and viral minor capsid proteins VP2 and VP3. Once the genome appears in the nucleus, early viral antigens are produced and mediate suitable environment for replication of viral genomes. After replication of viral DNA and morphogenesis of virions, virus progeny is released from the cells during its lysis. The research presented in the first part of thesis focused on intracellular transport of MPyV and involvement of cytoskeletal networks during virus delivery to the ER. In particular, we investigated still unclear role of microtubules during virus trafficking in endosomes, and involvement of microtubular motors. We found that MPyV trafficking leading to productive infection does not require the function of kinesin-1 and kinesin-2, but depends on functional dynein-mediated transport along microtubules. Dynein was shown to mediate translocation of virions from peripheral often multicaveolar-like compartments to the early and late endosomes, and further virus trafficking to the ER. Despite microtubules were also found to mediate virus transport to recycling endosomes, these compartments were showed to be dispensable for productive infection. In the second part, research focused on properties of the minor capsid proteins VP2 and VP3. Minor capsid proteins were showed to be indispensable for delivery of viral genome into the nucleus. The affinity of both minor proteins to artificial membranes and ability of VP2 protein to perforate these membranes were demonstrated. These finding thus suggest that the internal minor capsid proteins, exposed after capsid disassembly in the ER, might contribute to translocation of viral genome from ER to nucleoplasm. We prepared fusion variants of minor capsid proteins by linking them to enhanced green fluorescent protein (EGFP) and tested them during their individual expression in the absence of other MPyV gene products. Our biochemical studies proved each of the minor proteins to be a very potent inducer of apoptosis. Confocal and immunoelectron microscopy analyses showed ability of both minor proteins to interact with and damage the intracellular membranes, suggesting the mechanism of their cytotoxicity. Nevertheless, further analysis of apoptotic markers and cell death kinetics in cells transfected with MPyV genome mutated in both VP2 and VP3 translation start codons revealed that the minor proteins are only moderate contributors to apoptotic processes during infection and dispensable for cell destruction at the end of the virus replication cycle. These results thus indicate the role of the minor capsid proteins preferentially during virus entry and delivery of viral genome into the cell nucleus.